TRPC Channels

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TRPC1 Channels Mediate the Nonselective Cation Current and Store-Operated Calcium Channels in Human Atrial Myocytes

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Little information is available in the literature regarding transient receptor potential (TRP) channels in human atrial myocytes. The present study was designed to investigate whether TRPC channels would mediate the nonselective cation current reported previously and store-operated Ca^{2+} entry channels (SOCs) in human atrial myocytes using approaches of whole-cell patch voltage-clamp, RT-PCR, Western blot analysis, confocol microscopy, and co-immunoprecipitation. It was found that the nonselective cation current was recorded under K^{+}-free conditions in human atrial myocytes, and the current was inhibited by the nonspecific TRP channel blocker La^{3+}. The TRPC1 channel activator thapsigargin activated the current, and the effect was suppressed by La^{3+} and prevented by pipette inclusion of anti-TRPC1 antibody. In addition, confocol microscopic experiment revealed intracellular Ca^{2+} transient mediated by SOCs in human atrial myocytes, which was inhibited or prevented by La^{3+}. The mRNAs and proteins of STIM1 and Orai1 (components of SOCs), were abundantly expressed in human atria. Co-immunoprecipitation analysis demonstrated an interaction of TRPC1 with STIM1 and/or Orai1. Interestingly, protein expression of TRPC1 and STIM1, but not Orai1, was upregulated in human atria with atrial fibrillation. Our results indicate that the novel information that TRPC1 channels not only mediate the nonselective cation current, but also is a component of SOCs in human atria. The upregulation of TRPC1 and STIM1 in human atria with atrial fibrillation may suggest that TRPC1 channels and SOCs are likely involved in the atrial electrical and/or structure remodeling in patients with atrial fibrillation.

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TRPC3 Expression Modulates Store-Operated Currents in RBL-2H3 Cells

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TRPC3 was repeatedly discussed as a potential component of store-operated signaling pathways and is expressed in mast cells, which display the classical Orai1-mediated CRAC conductance. To elucidate if TRPC3 interferes with this store-operated conductance, we overexpressed TRPC3 in RBL-2H3 mast cells and characterized currents activated by passive store depletion (20mM EGTA). TRPC3 overexpression diminshed the inwardly rectifying CRAC current component significantly when free intracellular Mg^{2+} was kept at a level of 1.6 mM, and this inhibitory effect was more pronounced when free intracellular Mg^{2+} was elevated to 8 mM. Moreover, store depletion-induced currents were completely abolished with expression of a pore-dead mutant of TRPC3 (E630K). By contrast, the potential STIM-binding deficient mutant, E697K/E698K lacked effects on CRAC current amplitude. Importantly, at low (1.6 mM) intracellular Mg^{2+}, we observed a reduced Ca^{2+} selectivity of the store depletion-activated conductance with appearance of a clearly outward rectifying I-V relation. This nonselective conductance was absent at high (8 mM) Mg^{2+}, eliminated by co-expression of the permeation deficient Orai1 E106Q mutation and displayed sensitivity to the TRPC3 blocker Pyr3 (3 μM) as well as to the TRPM7 inhibitor NDGA (10 μM). Biophysical and pharmacological features of the nonselective conductance, which was activated by store depletion at low (physiologic) intracellular Mg^{2+} levels, favor the concept of generation of a unique membrane conductance by TRPC3 overexpression in RBL cells. Our findings suggest a complex interaction of TRPC3 with Orai1 and probably also TRPM7 in plasma membrane microdomains of mast cells.
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TRPC3 Overexpression Promotes AngiotensinII Induced Cardiac Dysfunction

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TRPC3 was recently suggested as a player in the development of cardiac hypertrophy. Little is known about the direct proarrhythmogenic role for TRPC3. Here we examined the involvement of TRPC3 in cardiac actions of AngiotensinII (AngII), a pathophysiologically relevant mediator and activator of GPCR/Gq/TRPC3 signaling, using a TRPC3 transgenic mouse model. A comparison of AngII actions in the isolated Langendorff perfused heart of TRPC3+/- (N=5) and wild-type (WT) mice (N=5) revealed that TRPC3 overexpression strongly promoted the impairment of cardiac functions by AngII. Administration of AngII (100nM) reduced left ventricular pressure (LVP) within 2 min to 64 %, + dP/dt to 50 % and - dP/dt to 55 % of control in TRPC3+/- hearts, while these functions remained largely unaffected in WT hearts. Simultaneously ECG recordings demonstrated AngII-induced episodes of acute arrhythmogenicity with ventricular ectopies in all TRPC3+/- hearts (N=6), whereas rhythm of WT hearts (N=6) remained unaffected. Changes in Ca2+ transients and sarcomere shortening were analyzed in isolated ventricular myocytes. AngII (100nM) induced a rise in the diastolic Ca2+ level, which was accompanied by irregular contractions in TRPC3 overexpressing but not in WT myocytes. Our results demonstrate that AngII modulation of cardiac functions is strictly dependent on TRPC3 expression and suggest a key role of TRPC channels in AngII-mediated arrhythmogenicity. Supporteded by FWF, DK-MCD

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TRPC3 Channel Blockers but not TRPC6 Blockers Inhibit Background Calcium Influx and Modulate Calcium Transients in Mouse Muscle Fibers

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We have recently shown that the cation channels TRPC3 and C6 are expressed in mouse skeletal muscle. Both mRNAs could be detected by RT-PCR and immunohistochemical staining revealed the presence of TRPC6 and in part TRPC3 in the sarcolemma of mouse muscle fibers. OAG, an activator of TRPC3/C6 and C7, stimulated background Ca^{2+} influx, supporting the hypothesis of functional expression of TRPC3 and/or C6 in skeletal muscle. TRPC6 could be pharmacologically activated, however, it does not seem to contribute to background Ca^{2+} influx, since the specific TRPC6 inhibitor ML-9 was ineffective. Here we studied whether the unspecific TRPC channel blocker 2-APB and/or the specific TRPC3 blocker Pyr3 affect muscular Ca^{2+} homeostasis. To investigate divalent cation influx we used single interosseus muscle fibers and applied the Mn^{2+} quench technique. Quench of Fura-2 fluorescence was recorded in the presence of 0.5 mM Mn^{2+} (excitation at 360 nm). Changes of cytoplasmic Ca^{2+} were measured using Fura-2 and alternate excitation at 340 and 380 nm. 2-APB inhibited background Ca^{2+} influx by more then 50% (n=44, $p<0.01$). The half time of decay of KCl induced calcium transients was as well significantly influenced by 2-APB (control vs. 2-APB; 3.61 ± 0.18 s vs. 3.18 ± 0.15 s; n= 27, 31; $p < 0.05$). The application of Pyr3 resulted in a marked inhibition of Fura-2 quench rate (control vs. Pyr3; 6.4 ±0.6 vs. 2.5 ± 0.4 %/min; n=46, 46; $p<0.01$). We conclude that both channels, TRPC3 and TRPC6 are functional in the sarcolemma of isolated mouse muscle fibers. However, TRPC6 has no resting activity, but TRPC3 contributes substantially to the background Ca^{2+} influx of muscle fibers.

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A Self-Limiting Regulation of TRPC3 C6 C7 Channels Linked with PI(4,5)P_2-Diacylglycerol Signaling

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TRPC3/C6/C7 channels are activated by diacylglycerol (DAG) upon the phospholipase C (PLC) coupled receptor stimulation which results in the breakdown of phosphoinositides (PIPs). Although the critical function of PIPs on various ion transporting molecules has been demonstrated, their roles in TRPC3/C6/C7 channels remain controversial. Here, by ectopical usage of voltage-sensing PIPs phosphatase (DrVSP), we found that dephosphorylation of PIPs produces robust inhibition of currents induced by carbachol (CCh), OAG and RHC80267, for all TRPC3/C6/C7 channels. The strength of inhibition was, however, variable with the rank order of DrVSP-mediated inhibition (VMI): C3 < C6 < C7. Pharmacological and molecular interventions most likely suggest that depletion of $PI(4,5)P_2$ is a critical event for VMI of all three channels. As predicted, when PLC catalytic signal was vigorously activated through overexpressed muscarinic type-I receptor (M1R), macroscopic kinetics of TRPC currents were greatly accelerated as similar the rank order of VMI, and it was simultaneously dismissed or attenuated. Likewise, VMI was rarely detected in vasopressin-induced TRPC6-like currents from smooth muscle cells (A7r5), supporting a severe or various degree of $PI(4,5)P_2$ depletion in the physiological conditions. We also employed simultaneous detection of FRET-based measurement of $PI(4,5)P_2$ and TRPC6 current, confirming that VMI reflects the degree

of PI(4,5)P_2 depletion. These results thus demonstrate that TRPC3/C6/C7 channels are differentially regulate by depletion of PI(4,5)P_2, and bimodal signal produced by PLC activation (i.e. depletion of PIP_2 and production of DAG) simultaniously controls these channels in a self-limiting manner.

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Selective $G\alpha_i$ Subunits as Novel Direct Activators of TRPC4 and TRPC5 Channels

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The Transient Receptor Potential, Canonical (TRPC) channels function as non-selective, Ca^{2+}-permeable channels and mediate numerous cellular functions. It is commonly assumed that TRPC channels are activated by stimulation of $G\alpha_q$ coupled receptors. However, whether the $G\alpha_q$-PLC pathway regulates the TRPC4/5 channels and how these channels are regulated by other $G\alpha$ proteins is unknown.

Here, we discovered that $G\alpha_i$ subunits, rather than $G\alpha_q$, are the primary and direct activators of TRPC4 and TRPC5. These channels were activated by the stimulation of Muscarinic receptor 2 that regulated by *pertusis* toxin sensitive manner in the activation process of channel.The expression of the constitutively active $G\alpha_i$ mutants selectively activates TRPC4 and TRPC5 channels. TRPC4 is activated by several $G\alpha_i$ subunits, most prominently by $G\alpha_{i2}$ and TRPC5 is activated primarily by $G\alpha_{i3}$. On the other hand, to investigate the effect of $G\beta\gamma$ on the activation process of TRPC4/5, we used $G\beta$ mutants ($G\beta_1^{W99A}$ and $G\beta_1^{I80A}$). The result from these mutants does not suggest the role of $G\beta\gamma$ subunit as a key modulator for TRPC4/5 activation. Finally, to check out that the mechanism of TRPC4 activation by $G\alpha_{i2,}$ we expressed TRPC4 C-terminus deletion and truncation mutants in HEK293 cells. When the region from 700 to 720 in C-terminal region of TRPC4 channel was deleted, electrophysiological activity did not elicited by $G\alpha_{i2}$ QL and infused GTPγS. Also co-IP between TRPC4 and $G\alpha_{i2}$ QL was altered by the deleted c-terminal region (700-720).These findings indicate an essential role of $G\alpha_i$ proteins as novel activators for TRPC4/5 and reveal the molecular mechanism by which G proteins activate the channels.

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Closely Spatio-Association of TRPC4 with $G\alpha_I$ in the TRPC4 Activation Process

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Canonical transient receptor potential (TPRC) channels are Ca^{2+}-permeable nonselective cation channels that are widely expressed in numerous cell types. Seven different members of TRPC channels are isolated and canonical type of TRP channel family transduces signals of GPCR with various external stimuli. TRPC4 channels are known to be regulated by $G\alpha i$ proteins. However, the molecular mechanism how $G\alpha i$ proteins activate TRPC4 still remains to be questionable. To investigate the mechanism, we used whole patch clamp and FRET (FluorescenceResonance Energy transfer). We tagged mTRPC4 and G protein with CFP and YFP, respectively, and transiently transfected HEK293 cells with FRET pair. FRET efficiency between TRPC4 and $G\alpha$ was 8.08 2.24% (n = 11) and was greater than those between TRPC4 and $G\beta\gamma$ (4.00 1.67 (n = 11)). At the HEK293 cell transfected with M2 muscarinic receptor, application of carbachol (CCh) increased FRET efficieny from 9.66 4.64 % (n = 7) to 26.27 10.09 % (n = 7). Intracellular 0.2mM GTPγS also increased FRET efficiency and TRPC4 current. In conclusion, we suggest that $G\alpha i$ closely locates near TRPC4 and regulates TRPC4 channel activity.

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Gs Regulates TRPC5 through IP_3 Mediated Ca^{2+} Release

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Canonical type of TRP channels have been reckoned as a molecular candidate for Ca^{2+}-permeable, nonselective cation channels which are ubiquitously expressed in mammalian cells. While there are various already-reported regulators both *in vivo* and *in vitro*, little is known, however, how heterotrimeric G-protein mediates external stimuli into TRPC5 function. We previously reported that Gs negatively regulates TRPC5 via direct phosphorylation by PKA and here, we demonstrate novel regulatory pathway regarding TRPC5 of which Gs downstream molecules govern through IP_3-mediated Ca^{2+} release. We performed various drug administrations during whole-cell patch clamp recording in the hTRPC5-transiently expressing HEK293 cells. Confocal Laser Scanning Microscopy was aided in addition to patch-clamping to measure membrane portion of TRPC5. We found that TRPC5 whole-cell current was increased by β-adrenergic receptor specific agonist, isoproterenol (246 ± 36%), adenylyl cyclase activator, forskolin (273 ± 6%), and membrane permeable analogue of cAMP, 8-Br-cAMP (232 ± 14%), whereas translocation onto plasma membrane was not the case. To assert the possibility of mediating Ca^{2+}-release, we infused inositol triphosphate (IP_3) in pipette solution which diminished potentiating effect of the drugs. In addition, population Ca^{2+} imaging showed robust Ca^{2+} release after drug treatment. Altogether, we conclude that Gs pathway regulates TRPC5 via not only inhibitory PKA phosphorylation but also activating Ca^{2+} release.

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Chemical Modification of Cysteine Residues Activates TRPC5 Channels

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The crucial cysteine residues can be involved in modulation of protein activity via modification of their thiol (Sulfhydryl; -SH) groups. These reactions can take several forms, such as redox events (chemical reduction or oxidation) or S-nitrosylation. However, regulation of transient receptor potential (TRP) channels by nitrosylation or breaking a disulfide bridge by thioredoxin remains unclear. Electrophysiological experiments reveal that TRPC5 is activated not olny several oxidants (DTNP, 2-PDS, DTNB) but also some reductants (DTT). But When cell-permeable DTT or cell-impermeable TCEP treated after DTNP application, only DTT inhibited the TRPC5 current activated by DTNP. And when containing Glutathione in pipette solution, the activating effect of DTNP obviously diminished in TRPC5. And we used the mutants of multiple cysteines on the intracellular amino and carboxyl terminus of TRPC5. The mutation of specific cysteines in TRPC5 channel markedly reduced current by DTNP. Also, we identified cytosolic cysteine sites to be modified using a prediction software.

In conclusion, we suggest that the chemical modification of intracellular cysteine is easy to activates TRPC5 in pathologic condition, such as low glutathione concentrations in cytosol. And TRPC5 may be activated by hypoxia through mechanisms involving cysteine oxidation.

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Differential Modulation of Detergents on Single Channel Activity of TRPC4/C5

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The effects of detergents on ion channel modulation have been studied before. Specifically, it has been noted that detergents used in the preparation of subunits of heterotrimeric G proteins can differentially modulate channel activity. A detailed analysis of the modulation, however, has not been performed. Here we show that detergents typically used in G protein subunit preparations, in sub-critical micelle concentrations, can affect the ion channel under consideration. TRPC4 and TRPC5 are closely homologous members of the Canonical Transient Receptor Potential family of non-selective cation channels. Activation of TRPC4 and TRPC5 elicits membrane depolarization and intracellular calcium signaling in neurons, vascular endothelium and smooth muscle cells. Both channels have been previously shown to be synergistically regulated by G_q-coupled receptor pathways and pertussis toxin sensitive $G_{i/o}$-coupled receptor pathways. The zwitterionic detergent CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate, 14-50 μM) and nonionic surfactant lubrol ($C_{12}E_{10}$, 0.001-0.004%), which are typically used in G protein subunit preparations, were applied to the cytosolic side of inside-out membrane patches excised from HEK293 cells expressing TRPC4 or TRPC5. Although both CHAPS and lubrol caused ~40-60% reduction in P_{open} under basal non-stimulated conditions, only CHAPS resulted in an increase in frequency of transitions between the open and closed states of the single channel activity. Therefore, the effects of detergents, especially at a single channel resolution, are